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Liquid Phase Piezoelectric Immunosensors

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Ahmad Suleiman
Chemistry Department
Southern University
Baton Rouge - LA 70813

SUMMARY

Piezoelectric crystal sensors offer excellent sensitivity and design simplicity that make them suited for space technology. The overall objective of the present research study is to develop a piezoelectric immunosensor for the determination of E. coli bacteria. However, the specific objectives of the first year are 1) to design and evaluate several oscillator circuits for liquid phase measurements, 2) to evaluate antibody immobilization procedures for optimal response characteristics.

The following tasks were accomplished during the first year. Two different methods for immobilizing the antibody on the piezoelectric crystals were tested. The first method involves use of protein A as a precoating of the gold electrode of the quartz crystal followed with coating with antibody. The second technique is immobilization of the antibody on the crystal surface via glutaraldehyde cross-linking using bovine serum albumin. Preliminary results have indicated that both methods proved to be suitable to measure E. coli. Linear calibration curves in the range of 7×10^4 to 7×10^7 Cell/mL and 7×10^5 to 7×10^7 Cell/mL were obtained with protein A and glutaraldehyde methods, respectively.

In addition, to facilitate liquid phase measurements, three oscillator circuits as described in the literature and a forth circuit designed by us were constructed. The respective circuit allows continuous measurements while the crystal is totally immersed in solution. In addition, it simultaneously allowed for dual monitoring of a reference and a sample crystal. The performance of these circuits was evaluated with respect to the effect of temperature and solution viscosity on the crystal frequency.

Table of Contents

SECTION 1.0 INTRODUCTION

The use of piezoelectric devices as potential sensor is attributed to the Sauerbrey equation:

$\Delta F = - 2.3 \times 10^{-6} F^2 \Delta M/A$

Where ΔF is the change in the frequency due to coating (Hz), F is the resonant frequency of the piezoelectric crystal (MHz), A is the area coated (cm^2) and ΔM is the mass deposited(g).

The reaction of the adsorbed coating placed on the crystal with an analyte causes a change in the frequency of the crystal providing qualitative and quantitative information about the respective analyte.

The use of antibodies as coating for the detection and determination of E. coli is investigated. Poor reproducibility normally associated with piezoelectric sensor can be addressed by improving the oscillator circuitry to enable continuous monitoring in liquid phase. The performance of a recently designed circuit in our laboratory seems to be very promising and a noticeable improvement in reproducibility has been achieved.

SECTION 2.0 OBJECTIVES

The overall objective of the present research study is to develop a piezoelectric immunosensor for the determination of E. coli bacteria. However, the specific objectives of the first year are:

- to design and evaluate several oscillator circuits for liquid phase measurements,
- to evaluate antibody immobilization procedures for optimal response characteristics

SECTION 3.0 EXPERIMENTAL

3.1 MATERIALS AND METHODS

Positive control E. coli bacteria was obtained from Kirkegaard & Perry Lab. Inc. Gaithersburg, MD (bacteria concentration 7×10^9 cell /mL.) The bacteria was rehydrated by adding 1 mL of deionized water. A working stock solution was prepared by diluting

0.1 mL of the concentrated solution to 10 mL with phosphate buffer (pH 7.0). Stock bacteria solution was kept in the freezer. Fresh working standards were prepared when needed.

Affinity purified antibody to E. coli, isolated from a serum pool from goats immunized with whole cells of E. coli was obtained from Kirkegaard & Perry Lab. Inc. Gaithersburg, MD. To each vial 0.2 mL of phosphate buffer (pH 7.0) was added to give a final concentration of 5 mg/mL. The antibody solution was kept at 4 °C.

Glutaraldehyde (25 % aqueous solution) was obtained from Sigma Chemical Company, St. Louis, MO. Different concentrations of glutaraldehyde were prepared by diluting the stock solution with deionized water.

Protein A was obtained from Sigma Chemical Company, St. Louis, MO. A stock solution of protein A was prepared by dissolving 5.4 mg in 1 mL of phosphate buffer (pH 7.0.)

Bovine serum albomin was obtained Sigma Chemical Company, St. Louis, MO.

3.2 MEASURING EQUIPMENT

The piezoelectric crystals AT-cut with a basic resonance frequency of 10 MHz (Bliely Electric Co.) Frequency measurements were made using either a frequency counter model PZ 106, Universal Sensors, or a Heath digital frequency counter SM-2420 equipped with a Heath 2718 tri-power supply.

3.1 IMMOBILIZATION TECHNIQUE

Two techniques to immobilize the antibody on the surface of the quartz crystal were investigated.

3.1.1 Immobilization via protein A

Before use, the leads of the quartz crystals were coated with a commercial wax to avoid leads rust during successive dipping in aqueous solution. The quartz crystals were washed in 1 M solution of NaOH for 10 minutes followed by another 10 min wash in 1 M HCl. The crystals were then washed with distilled water and air dried. The frequency of each crystal was measured. To each side of the gold electrodes of the crystal, 2.5 μL of protein A (5.4 mg/mL) was added. The protein A was allowed to dry for minimum period of two hours. The crystals were washed with phosphate buffer for 10 min followed with 10 min wash with deionized water. The crystals were dried with a gentle stream of air. The frequency was measured again. To each side of the gold electrode a 2.5 μL of E. coli antibody was spread, air dried, washed with phosphate buffer and deionized water as described before and allowed to dry, the crystal frequency was measured again.

3.1.2 Immobilization via glutaraldehyde, BSA cross-linking

New quartz crystals were washed and leads were wax treated as described before. To each surface of the gold electrode of the crystal, 2.5 μL of the E. coli antibody, 2 μL of bovine serum albumin (BSA) and 2 μL of glutaraldehyde were added, and the three components were mixed on the crystal surface using a glass rod. The mixture was allowed to air dry. The crystals were then incubated in a wet chamber for 1 hour. The crystals were washed with a 0.1M glycine/phosphate buffer solution (pH 7), to block the unreacted sites of the aldehyde groups. The crystals were finally washed with deionized water for 10 minutes, air dried and the frequency was measured.

3.1.3 MEASUREMENT PROCEDURE

Two techniques were followed to measure the frequency drop of the crystals:

3.1.3.1 The dry technique

The difference between the frequency of the antibody-coated crystal and the uncoated crystal was first determined ΔF1. The coated crystals were then dipped in bacteria solution of different concentrations for 30 minutes. Three crystals were used at each concentration level. Allowed to air dry, and washed with deionized water for 10 min and dried again before its frequency is measured. The frequency difference between the antibody-coated crystal and the bacteria-exposed crystals is determined ΔF2. The shift in the crystal frequency is related to the concentration of the bacteria solution.

3.1.3.2 The wet technique

In this technique, the antibody-coated crystals were not dried. The Frequency of the crystals was measured first directly in 0.01 M phosphate buffer solution. Then it was washed with deionized water. The crystal was then immersed in the bacteria solution prepared in 0.01 M phosphate buffer. The frequency measurements were made after 5 minutes to allow for signal stabilization

SECTION 4.0 RESULTS AND DISCUSSION

Three different frequency circuits were constructed in our laboratory as it has been described in the literature [1-3]. The performance of these circuits was evaluated with respect to; possibility of frequency measurements in solution; effect of temperature and solution viscosity on crystal frequency. A forth circuit was constructed by us and the same test was applied to it. The results of the study have shown that, the first three circuits were not able to measure the crystal frequency in solution. The first two circuits were able to give a reasonable results on one side of the crystal only, but the signal

disappears when the crystals were totally immersed in solution (deionized water or phosphate buffer pH=7). Although the third circuit was able to produce a measurable frequency, the frequency was not stable to allow a reliable measurement. The results obtained with our circuit however, produced very promising results.

4.1 EFFECT OF TEMPERATURE

The effect of solution temperature on the crystal frequency was investigated using our oscillator circuit. The results which show the effect of temperature on the crystal frequency are shown in Fig. 1. It can be seen that temperature has a very significant effect on the measurements, and suggest that the system temperature should be carefully controlled during the measurements.

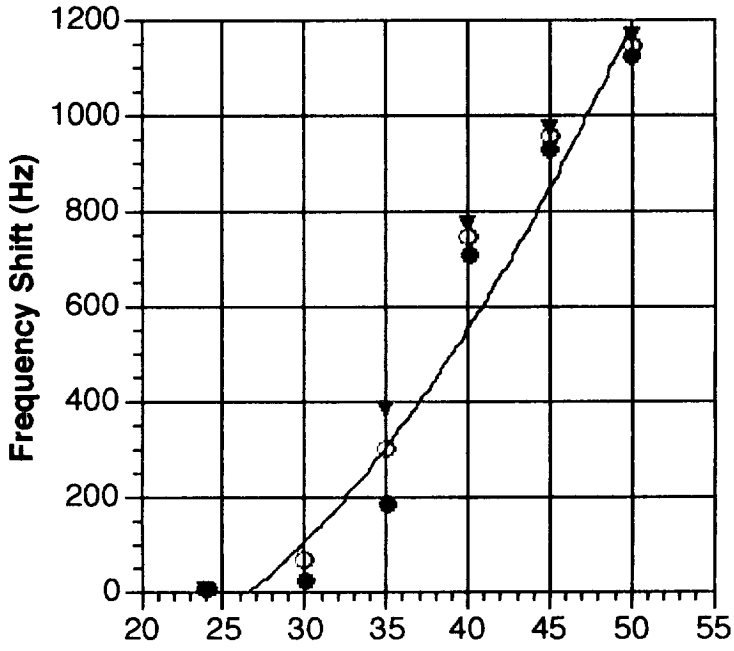

Figure 1. Effect of solution temperature on quartz crystal frequency

4.2 EFFECT OF VISCOSITY

The effect of viscosity on the frequency of the quartz crystal was investigated using dextrose solution in the range of 1.002η to 2.992η. A graph which represents this relationship is shown in Fig. 2. The experimental results shown in Fig 2, clearly demonstrate the importance of solution viscosity when measuring crystal frequency in solution.

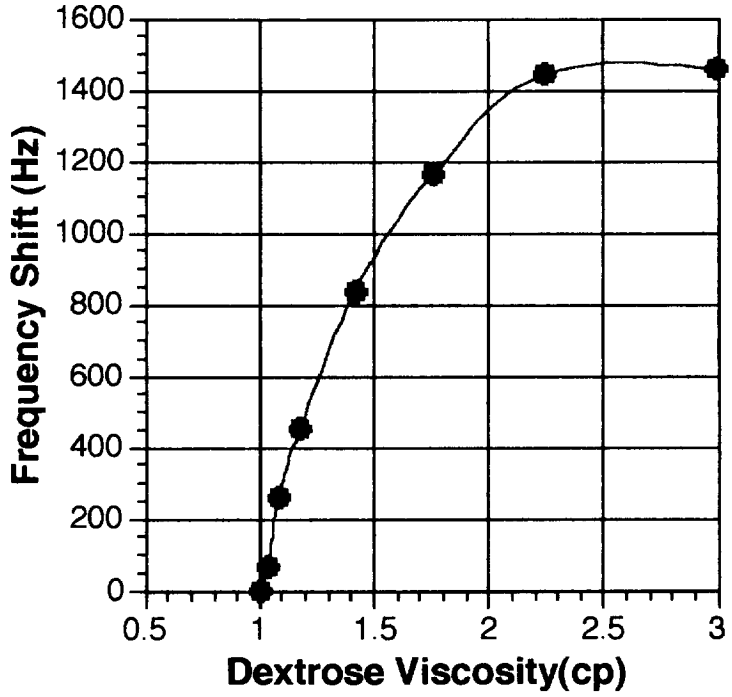

Figure 2. Effect of dextrose solution viscosity on quartz crystal frequency

4.3 STUDY OF IMMOBILIZATION TECHNIQUES

4.3.1 Immobilization via Protein A

Immobilization of the antibody with protein A gave the most reproducible results. However the frequency shift was generally small. A linear calibration curve in the range of 7 $X10^4$ to 7 X 10^7 cell/mL was obtained for the determination of E. coli bacteria in solution is shown in Fig 3.

4.3.2 Immobilization via Glutaraldehyde/BSA

The frequency shift obtained with this method was much higher than those obtained with protein A method. The higher signal could be due to larger amount of immobilized antibody on the electrodes of the crystal. However, the reproducibility was much less. The calibration was linear over the range of 7 x 10^5 to 7 X 10^7 cell/mL.

SECTION 5.0 FUTURE RESEARCH PLAN

During the second year, the selectivity, stability and lifetime of the coating will be studied. Since it seems that the sensitivity may be improved by increasing the amount of immobilized antibody, other new method such as electro-polymerization of the antibody

may be investigated. In addition, to improve the reproducibility, a thermally isolated flow cell with a reference and a sensing crystal will be constructed and used.

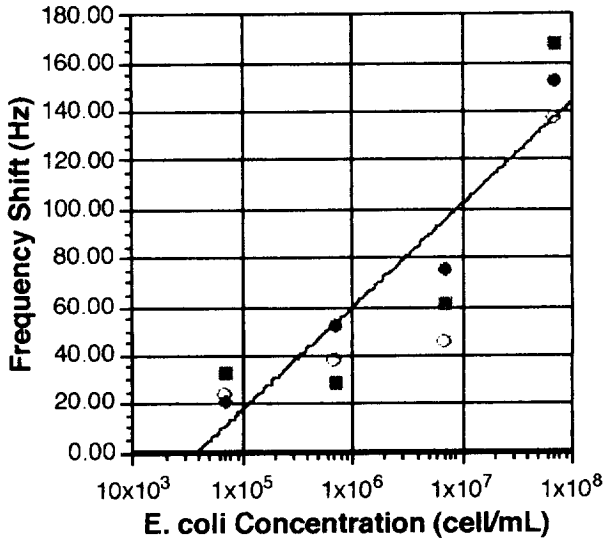

Figure 3. A calibration curve for the determination of E. Coli in solution using protein A immobilization method

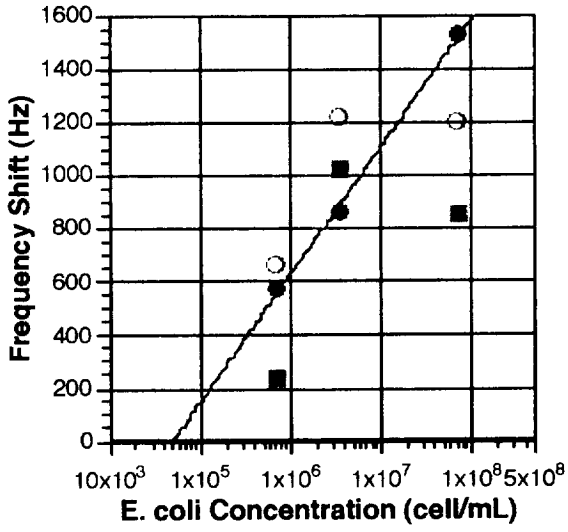

Figure 4. A calibration curve for the determination of E. Coli in solution using glutaraldehyde/BSA immobilization method

SECTION 6 REFERENCES

1- Kurosawa, E. Tawara, N. Kamo and Y. Kobatake, Anal. Chim. Acta 230, 41 (1990).

2- Immuno Biosensor , Pz-106 , Universal Sensor Inc. New Orleans.

3- Shou-Zhuo and M. Zhi-Wong, Anal. Chim. Acta 193, 97 (1987).